REMARKS

I. Status of the Claims

Claims 67 - 86 are pending.

II. The Amendments

None of the amendments introduces new matter.

The specification has been amended to refer to sequence identification numbers. The specification has also been amended to recite the figure number next to that of the figure's panels. Support for the amendment to page 39 is found, *inter alia*, at specification page 14 (legend to Figure 1).

New claims 67-86 have been added without adding new matter. Support for the new claims is found, *inter alia*, in the claims as filed.

Additional support for "introducing" the DNA into a mammalian cell as in claim 67 is found, *inter alia*, at specification page 27, lines 24-25.

In claims 68 and 69, additional support for DNA fragments that are either ligated to each other or not ligated to each other is found, *inter alia*, at specification page 25, lines 17-20.

Additional support for claim 70 is found, *inter alia*, at specification page 11, lines 23-24.

Additional support for claim 71 is found, *inter alia*, at specification page 32, lines 18-20.

Additional support for claim 72 is found, *inter alia*, at specification page 12, lines 16-21.

Additional support for claim 73 is found, *inter alia*, at specification page 17, last paragraph, and page 27 lines 9-23.

Additional support for claim 74 is found, *inter alia*, at specification page 19, lines 7-8.

Additional support for claim 75 is found, *inter alia*, at specification page 12, lines 16-21.

Additional support for claim 76 is found, *inter alia*, at specification page 13, lines 13-16 and page 26, lines 1-6.

Additional support for claim 77 is found, *inter alia*, at specification page 29, lines 14-15, and pages 39-40.

Additional support for claim 78 is found, *inter alia*, at specification page 14, lines 10-12.

Additional support for claim 79 is found, *inter alia*, at specification page 20, lines 3-6 and also the last two paragraphs, page 11, lines 3-7, page 26 and page 12, lines 10-12.

Additional support for claim 80 is found, *inter alia*, at specification page 11, lines 23-24.

Additional support for claim 81 is found, *inter alia*, at specification page 32, lines 18-20.

Additional support for claim 82 is found, *inter alia*, at specification page 12, lines 16-21.

Additional support for claim 83 is found, *inter alia*, at specification page 26, lines 1-6.

Additional support for claim 84 is found, *inter alia*, at specification page 29, lines 14-15.

Additional support for claim 85 is found, *inter alia*, at specification page 14, lines 10-12.

Additional support for claim 86 is found, *inter alia*, at specification page 19, lines 7-8.

III. Summary

In view of the preliminary amendment and the above comments, it is believed that the present application is now in condition for immediate allowance. Early notice to this effect is earnestly solicited.

If, in the Examiner's opinion, a phone conference may expedite prosecution of this application, the Examiner is encouraged to contact Applicants' undersigned attorney at (202)371-2600.

Respectfully submitted,

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Version with markings to show changes made

The paragraph beginning at page 14, line 22 has been amended as follows:
(Italics are in the original text)

Figure 1. Method for producing large head-to-tail tandem arrays of alpha satellite DNA. pVJ104-Yα16 was linearized with *BamHI* and *SfiI*, and purified by pulsed field gel electrophoresis (PFGE). Likewise, pBac-Yα16 was linearized with *BamHI* and *BglII* and the alpha satellite array was purified by PFGE. Fig. 1A) The purified arrays were incubated together in the presence of ligase, *BamHI* and *BglII*. Since *BamHI* and *BglII* are complementary/ nonisoschisomeric overhangs, a ligation event resulting in a *BamHI/BglII* junction (as is the case in a head-to-tail joining) will destroy both sites. Thus, a head-to-tail junction will be resistant to cleavage by *BamHI* and *BglII*. In contrast, a head-to-head, or tail-to-tail ligation event will recreate a *BamHI* or *BglII* site, respectively. Since *BamHI* and *BglII* are present, these ligation products will be cleaved to produce their constituent monomers (or head-to-tail multimers). By controlling the amount of ligase, the incubation time, and the concentration of DNA, the length of the head-to-tail products can be varied as necessary. Fig. 1B) Following ligation, the products were analyzed by PFGE. Lane 1, molecular weight standards (NEBL Midrange II markers); lane 2, Yα16 (*BamHI/BglII* fragment) ligated in the presence of *BamHI* and *BglII* for 4 hours; lane 3, Yα16 (*BamHI/BglII* fragment) ligated in the presence of *BamHI/BglII* for 12 hours; lane 4, Yα16 (*BamHI/BglII* fragment) mock-ligated in the presence of *BamHI* and *BglII*; lane 5, VK75 (*BssHII* fragment) ligated for 12 hours without restriction enzyme; lane 6, VK75 (*BssHII* fragment) ligated for 12 hours in the presence of *BssHII*; lane 7, VK75 (*BssHII* fragment) mock-ligated. The molecular weight of ligation products are shown on the left. Note: Although these samples were run on the same gel, several irrelevant lanes between lanes 4 and 5 were removed.

The paragraph beginning at page 15, line 19 has been amended as follows:

Figure 3. Analysis of synthetic chromosomes from clones 22-7 and 22-13 by fluorescent *in situ* hybridization (FISH). Cells were harvested, dropped onto glass slides, and hybridized to Y alpha satellite DNA as described in the Experimental Procedures (See Examples herein). The biotinylated probe was detected using Texas Red Avidin and amplified with two layers of biotinylated anti-Avidin and Texas Red Avidin. Fig. 3A) DAPI image of a metaphase spread from clone 22-7. Fig. 3B) Same as Fig. 3A) except that the alpha satellite probe was visualized using a triple cube filter. Fig3C) DAPI image of a metaphase spread from clone 22-13. Fig. 3D) Same as Fig. 3C) except that the alpha satellite probe was visualized using a triple cube filter. In each case, the synthetic chromosome is indicated with a white arrow.

The paragraph beginning at page 16, line 3 has been amended as follows:

Figure 4. Analysis of synthetic chromosomes from clones 22-6 and 23-1 by FISH. Cells were harvested, dropped onto glass slides, and hybridized to Y alpha satellite DNA (clone 22-6) or 17 alpha satellite DNA (clone 23-1) as described in the experimental procedures. The biotinylated probe was detected using Texas Red Avidin and amplified with two layers of biotinylated anti-Avidin and Texas Red Avidin. Fig. 4A) DAPI image of a metaphase spread from clone 22-6. Fig. 4B) Same as A) except that the alpha satellite probe was visualized using a triple cube filter. Fig. 4C) DAPI image of a metaphase spread from clone 23-1. Fig. 4D) Same as Fig. 4C) except that the alpha satellite probe was visualized using a triple cube filter. In each case, the synthetic chromosome is indicated with a white arrow. [In D), the yellow arrow indicates the location of the C qter integration site.]

The paragraph beginning at page 16, line 14 has been amended as follows:

Figure 5. Analysis of synthetic chromosomes from clones 22-11 and 17-15 by FISH. Cells were harvested, dropped onto glass slides, and hybridized to Y alpha satellite DNA (clone 22-11) or 17 alpha satellite DNA (clone 17-15) as described in the experimental procedures. The biotinylated probe was detected using Texas Red Avidin and amplified with two layers of biotinylated anti-Avidin and Texas Red Avidin. Fig. 5A) DAPI image of a metaphase spread from clone 22-11. Fig. 5B) Same as Fig. 5A) except that the alpha satellite probe was visualized using a triple cube filter. Fig. 4C) DAPI image of a metaphase spread from clone 17-15. Fig. 5D) Same as Fig. 5C) except that the alpha satellite probe was visualized using a triple cube filter. In each case, the synthetic chromosome is indicated with a white arrow. In Fig. 5D), the yellow arrow indicates the location of the C qter integration site.

The paragraph beginning at page 17, line 1 has been amended as follows:

Figure 6. Determination of the amount of transfected alpha satellite DNA present in clones containing the synthetic chromosome. Fig. 6A) Total genomic DNA was harvested, digested, and electrophoresed as described in the Experimental Procedures. Lane 1, HT1080; lane 2, clone 22-6; lane 3, clone 22-7; lane 4, clone 22-11; lane 5, clone 22-13; lane 6, clone 23-1. Fig. 6B) The estimated amount of synthetic Y alpha satellite DNA is shown for each clone. Note: clone 23-1 was transfected with 17 alpha satellite DNA, and therefore, does not contain synthetic Y alpha satellite DNA.

The paragraph beginning at page 17, line 9 has been amended as follows:

Figure 7. CENP-E is associated with the synthetic chromosomes during mitosis. Immunofluorescence was carried out on metaphase chromosomes harvested from synthetic chromosome-containing clones as described in experimental procedures. Fig. 7A) DAPI-stained chromosomes from clone 22-11. Fig. 7B) Same as Fig. 7A) except the location of the

anti-CENP-E antibodies is visualized using a triple cube filter. Fig. 7C) DAPI- stained chromosomes from clone 23-1. Fig. 7D) Same as Fig. 7C) except the location of the anti-CENP-E antibodies is visualized using a triple cube filter. In each case, the synthetic chromosome is indicated by a white arrow.

The paragraph beginning at page 17, line 17 has been amended as follows:

Figure 8. X-Gal plate staining of clone 22-11 after growth for 70 days in the absence of selection. Cells were harvested and stained as described in the Experimental Procedures herein. Fig. 8A) HT1080 Fig. 8B) Clone 22-11. The presence of blue cells in clone 22-11, but not in HT1080 indicates that β-geo is still expressed in these cells.

The second full paragraph on page 21 has been amended as follows:
(Bold text is in the original)

Thus, in one embodiment of this invention, the centromeric DNA contains subregions within alpha satellite DNA. In a preferred embodiment, the centromeric DNA is composed of tandemly ligated CENP-B boxes, defined by the sequence 5′a**TTCG**ttgg**A**aa**CGGG**a3′ (SEQ ID NO:1), where the bases indicated by capital/bold letters are the most important for CENP-B binding and the bases indicated by lower case letters may be substituted with other bases.

The last paragraph on page 38 has been amended as follows:

Human telomeric DNA was generated by PCR using primers 42a (5′GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG3′) (SEQ ID NO: 2) and 42b (5′CCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCC3′) (SEQ ID NO: 3) (Ijdo, J.W. *et al., Nucleic Acids Res. 19*:4780 (1991)). Each PCR reaction contained 250 ng of 42a and 42b, 5 Units Taq polymerase, 250 μM dNTPs, 3.3 mM $MgCl_2$ in 1X PCR Buffer (Gibco BRL). The PCR reaction was carried out for 35 cycles in a Perkin Elmer 9600 Thermal cycler using the following temperature profile: 95°C for 20 seconds, 40°C for 20 seconds, 72°C for 2 minutes. Following PCR, each reaction was subjected to agarose gel electrophoresis to purify telomeric DNA that is greater than 1 kb in size. This DNA was excised from the gel and purified away from the agarose using Magic Prep columns according to the manufacturer's instructions (Promega, WI).

The paragraph beginning at page 39, line 2 has been amended as follows:
(Italics are in the original text)

Prior to transfection, pVJ105 Yα16 and pVJ105 17α32 were digested with *BamHI* and *SfiI*; pBac Yα16 and pBac Yα32 were digested with *BamHI* and *BglII*. The DNA was then purified by PFGE, equilibrated against 10 mM Tris pH 7.5, 100 mM NaCl, and combined with telomeric DNA and/or *NotI* digested human genomic DNA. In some cases, the alpha satellite arrays were extended using the directional ligation approach described in Figure 1A.

In the claims

Claims 2, 4, 6, 8, 10, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 41, 42, 44, 45, 47, 49, 51 and 59-66 have been canceled without prejudice or disclaimer.

New claims 67 - 86 have been added.